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TITLE: A Novel Approach to Monitoring Prostate Tumor Oxygenation: Proton MRI of the Reporter Molecule Hexamethyldisiloxane

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Growing evidence from experimental and clinical studies confirms that solid human tumors have foci of hypoxic cells, which have a profound influence on the therapeutic outcome of cancer chemotherapy and radiotherapy. A strong argument therefore exists for assessing the hypoxic fraction of tumors prior to patient treatment, and to tailor this treatment accordingly. It has been shown that there is linear relationship between R of hexamethyldisiloxane (HMDSO) and pO2, and the R1 of HMDSO is insensitive to various ions and minimally sensitive to temperature. The primary sequence for in vivo T1 measurment with water suppression has been established, and HMDSO is also detectable in prostate tumor. So, Hexamethyldisiloxane shows promise as a reporter molecule to measure tumor oxygenation by 1H MRS and potentially by MRI. This opens new opportunities for MR tumor oximetry, particularly since HMDSO is used widely in biomedical materials and as an ingredient in consumer products; and HMDSO is reported to have minimal toxicity.

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Introduction:

Growing evidence from experimental and clinical studies confirms that solid human tumors have foci of hypoxic cells, which have a profound influence on the therapeutic outcome of cancer chemotherapy and radiotherapy: the level and severity of hypoxia can be a strong prognostic factor of disease progression and survival. A strong argument therefore exists for assessing the hypoxic fraction of tumors prior to patient treatment, and to tailor this treatment accordingly.

Baseline pO_2 and dynamic changes with respiratory intervention has been measured extensively in rat prostate tumors by ^{19}F MRI(*FREDOM*, *F*luorocarbon *R*elaxometry using *E*cho planar imaging for *D*ynamic *O*xygen *M*apping) based on the spin-lattice relaxation of Hexafluorobenzene(HFB), which provides spatially resolved maps of tumor oxygenation at depth and allows monitoring of dynamic changes at specific locations. However, for clinical application ^{19}F NMR is not yet widely available. A proton MR analogue of HFB could facilitate immediate widespread oximetry. We have identified hexamethyldisiloxane(HMDSO) as a potential reporter. HMDSO has extensive symmetry and a single proton resonance well removed from water.

We had three specific aims for this project:

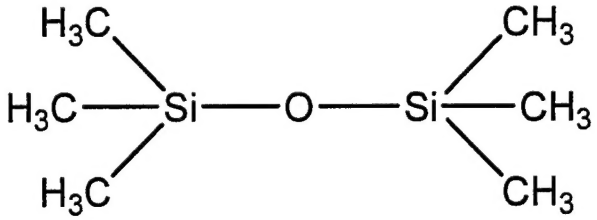
Task1. Characterize the effects of external factors on T1(spin-lattice relaxation time) of HMDSO in vitro and get the calibration curves, Month 1-6

Task2. Develop relevant MR pulse sequences with H_2O suppression for imaging. Establish experimental prostate tumor models and measure the retention time of HMDSO in tumors, Month 6-12

Task3. Make pO_2 maps and measure prostate tumor oxygen dynamics with respect to growth rate and respiratory challenge, Month 12-24

Body:

Hexamethyldisiloxane(HMDSO) is used widely in biomedical materials and as an ingredient in consumer products, such as a thin polymeric coating on suture for cardiovascular surgery, or the thin layer onto the inner surface of plasma-modified small diameter tubing, etc. HMDSO is reported to have minimal toxicity. A previous test, by me, in vitro indicates that the spin-lattice relaxation time of HMDSO is sensitive to oxygen tension. HMDSO is highly hydrophobic, and therefore can be injected intratumorally at specific sites and will not diffuse in the tumor. Moreover, the boiling point of HMDSO is 99-100°C. In addition, HMDSO only has one ^1H signal and the chemical shift difference between HMDSO and H_2O is about 4.7ppm. Thus, we believe HMDSO may be appropriate for measuring tumor pO_2 by MRS and MRI of ^1H. In this proposal, I plan to develop proton MR techniques using this new reporter molecule, HMDSO, to assess the prostate tumor oxygenation in different prostate tumor sublines with several selected levels of histological differentiations.



For the first year, I had the statement of work as:

Task1. Characterize the effects of external factors on T1(spin-lattice relaxation time) of HMDSO in vitro and get the calibration curves, Month 1-6

Measure the influence of

a. different temperatures on T1 of HMDSO
b. different metal ions on T1 of HMDSO
c. different B_0 on T1 of HMDSO
d. different pO_2 on T1 of HMDSO at various temperature, metal ions and B_0.

Task2. Develop relevant MR pulse sequences with H_2O suppression for imaging. Establish experimental prostate tumor models and measure the retention time of HMDSO in tumors, Month 6-12

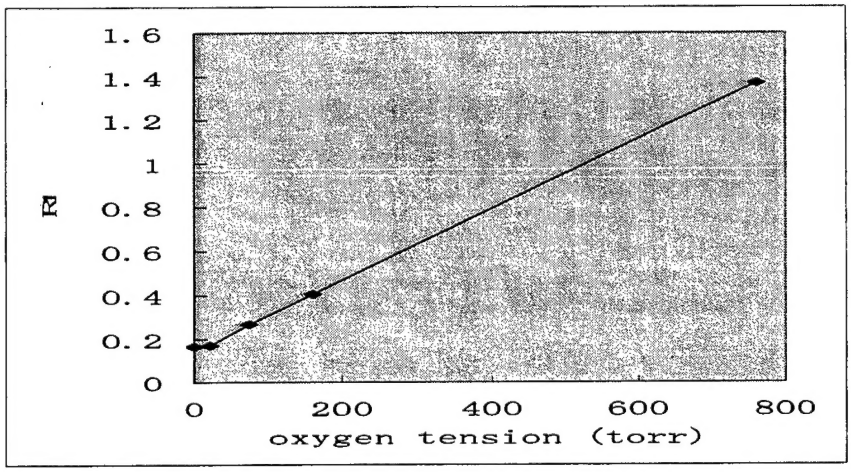
a. Become proficient with state of the art NMR e.g. echo planar imaging
b. Design and implement special pulse sequences to suppress the resonance of H_2O while meaning relaxation
c. Become proficient with surgically creating tumor pedicles and implantation of prostate tumors
d. Use three selected sublines of the Dunning prostate tumor in rats, allow to grow to 1 cm diameter and measure the retention time of HMDSO

Key research accomplishments:

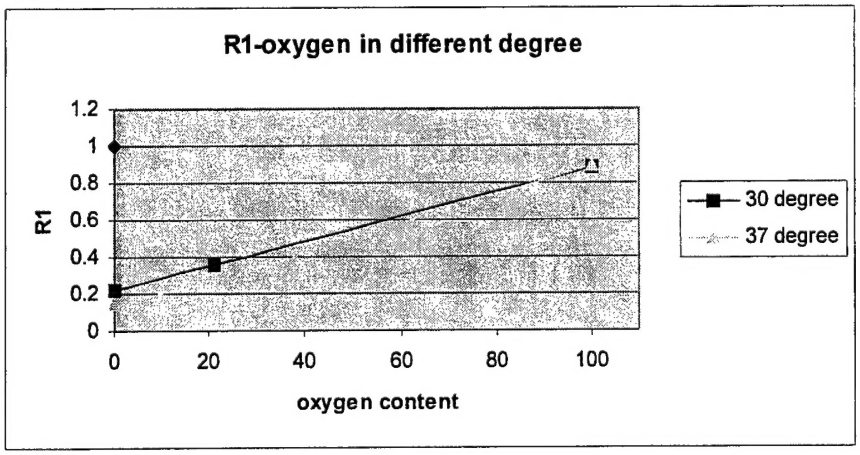
Task1. In order to characterize the effects of external factors on T1(spin-lattice relaxation time) of HMDSO in vitro and get the calibration curves, we measured the influence of: a). different ions on T1 of HMDSO, b). different temperatures on T1 of HMDSO, c) different B0 on T1 measurement, d) different pO2 on T1 measurement

1. T1 of HMDSO with different oxygen tension was measured on Varian 200 MHz and 600 MHz.

200MHz:



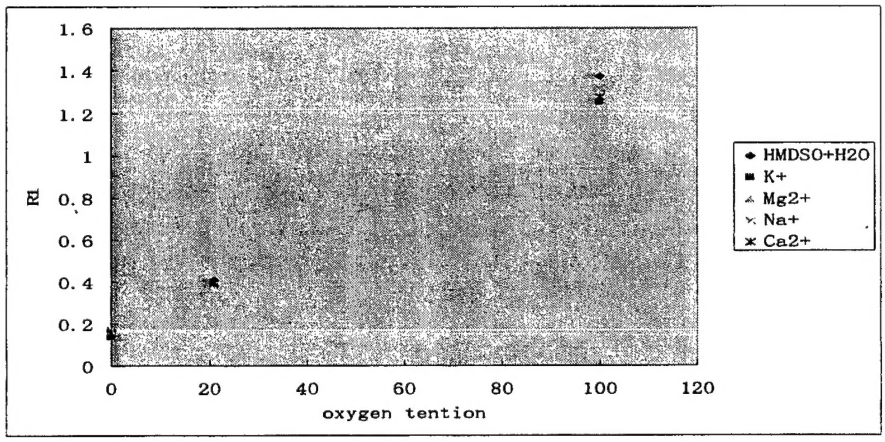
600 MHz

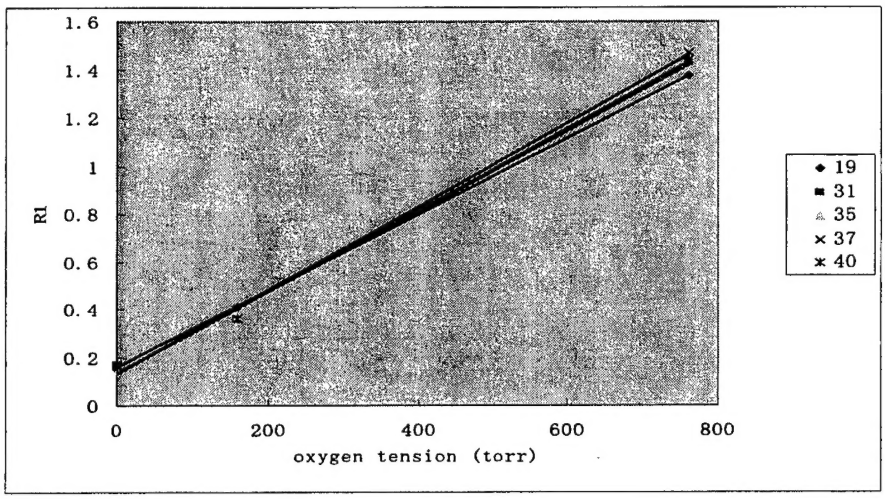


From above data, we can conclude that there is linear relationship between oxygen tension and spin-lattice relaxation rate. The correlation equation at 200 MHz is: R1=0.15+0.00168x (X is oxygen tension (torr).

2. Prepare the solution of Na^+, K^+, Mg^{2+}, Ca^{2+}, the mixture of HMDSO+H2O, and the ion concentration is calculated according to the proportion of various ion in vivo. The

T1 of these solutions were measured on 200MHz with various temperature and oxygen tension. It was found that there is no significant influence of various ions on spin-lattice relaxation rate, especially in the condition of lower oxygen tension that tumors have. R1 of HMDSO is only minimally sensitive to temperature. The correlation equation at 37°C is R1=0.13+0.00175x (X is oxygen tension (torr).





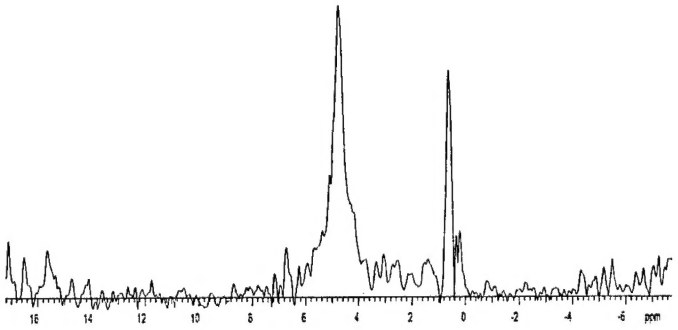
Task2. Develop relevant MR pulse sequences with H_2O suppression for imaging. Establish experimental prostate tumor models and measure the retention time of HMDSO in tumors

a. I become proficient with surgically creating tumor pedicles and implantation of prostate tumors

For thigh tumor: a flap of depilated skin is raised from the thigh of young adult male Copenhagen rat and held in position. A 1cm incision is made. A piece of fresh tumor tissue ($2x2x2mm^3$) is implanted under the skin and the cut closed with a wound clip.

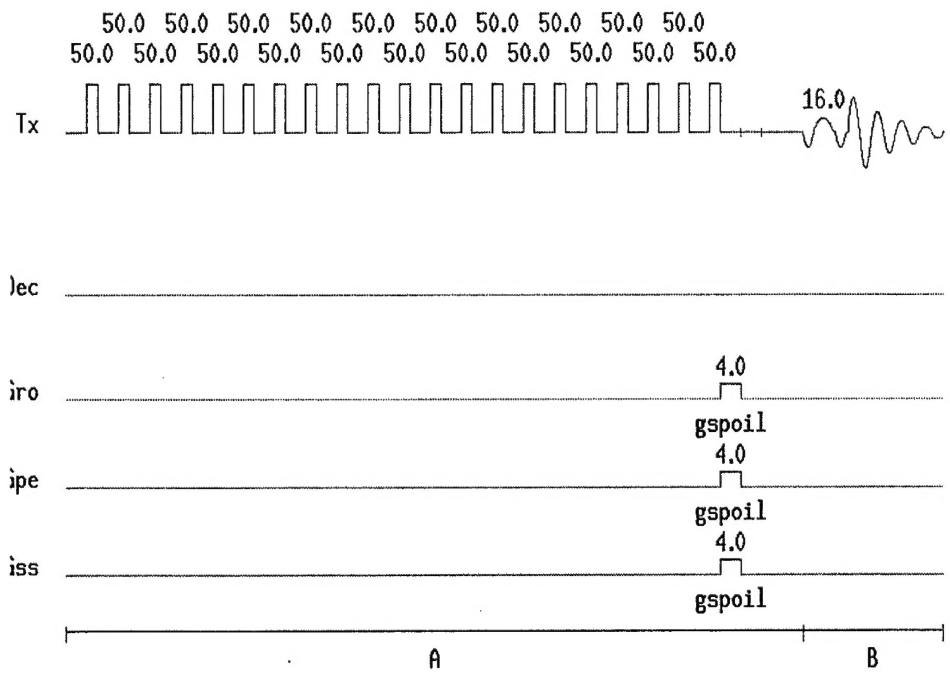
For pedicle tumor: A flap of depilated skin is raised from the body of young adult male Copenhagen rat and held in position with a non-traumatic curved bull-dog clip. A 3cm incision is made through the skin using the curved edge of the clip as guide. Would clips are used to join the edge of the skin producing a tube resembling a suitcase handle. Two weeks later, the clips are removed and the distal end of the pedicle severed. A piece of fresh tumor tissue ($2x2x2mm^3$) is implanted in the lumen and the cut closed with a wound clip.

b. I used selected sublines of the Dunning prostate tumor in rats to measure the retention time of HMDSO in tumor. 100 μl HMDSO was injected intratumorally into Dunning prostate 3327 AT1 and H tumor, and PRESS pulse sequence with water suppression was used to acquire data. It was shown that HMDSO can stay at least 24hr within prostate tumor.



c. We design and implement special pulse sequence to suppress the resonance of H2O while meaning relaxation. This work supported by MR scientist Dr. Vikram Kodibagkar.

For T1 measurements by spectroscopy, a pulse burst saturation recovery (PBSR) sequence consisting of 20 nonselective 90° pulses for saturation of signal followed by a delay tau for magnetization recovery and a single, frequency selective 90° pulse for signal detection was used. T1 values were measured using this sequence with the ARDVARC (Alternating Relaxation Delays with Variable Acquisitions for Reduction of Clearance effects) protocol. For imaging experiments a spin-echo EPI based PBSR pulse sequence is being designed for measuring T1 values. The sequence will consist of a) a train of 20 non-selective 90° pulses for saturation of signal followed by a delay tau for magnetization recovery, b) three optional CHESS pulses for frequency selective saturation of residual water and fat immediately followed by c) spin-echo EPI detection with a slice selective 90° pulse and a frequency selective 180° pulse.



Reportable outcomes:

One paper describing work supported by this grant will be presented as poster at the International Society of Magnetic Resonance in Medicine on May 2004.

Hexamethyldisiloxane (HMDSO), a novel reporter molecule for in-vivo oximetry using 1H MRI., Vikram D. Kodibagkar, Matthew E. Merritt, Weina Cui, Ralph P. Mason. ISMRM, May 15, 2004, Kyoto, Japan

Conclusions:

The above experiments showed that: 1) there is linear relationship between R1 of HMDSO and pO_2; 2) the R1 of HMDSO is insensitive to various ions and minimally sensitive to temperature; 3) the correlation equation at 37°C is R1=0.13+0.00175x (X is oxygen tension (torr),; 4) HMDSO is detectable in a tumor, and it remains present in tumor more than 24hrs; 5) the primary sequence for in vivo T1 measurement with water suppression has been established. So, Hexamethyldisiloxane shows promise as a reporter molecule to measure tumor oxygenation by ^{1}H MRS and potentially by MRI. This opens new opportunities for MR tumor oximetry, particularly since HMDSO is used widely in biomedical materials and as an ingredient in consumer products; and HMDSO is reported to have minimal toxicity.

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Appendix 1

Hexamethyldisiloxane (HMDSO), a novel reporter molecule for in-vivo oximetry using 1H MRI. Vikram D. Kodibagkar, Matthew E. Merritt, Weina Cui, Ralph P. Mason. Department of Radiology, UT Southwestern Medical Center, Dallas, TX

Synopsis

We present here, a proof of principle for the use of hexamethyldisiloxane (HMDSO) as a novel MRI based oximetry probe. The presence of water and fat resonances is the chief hurdle in imaging and relaxometry of a proton based oximetry probe. A spin-echo EPI based imaging protocol is presented with water and fat suppression for potential in vivo relaxometry applications. Results of phantom studies are reported.

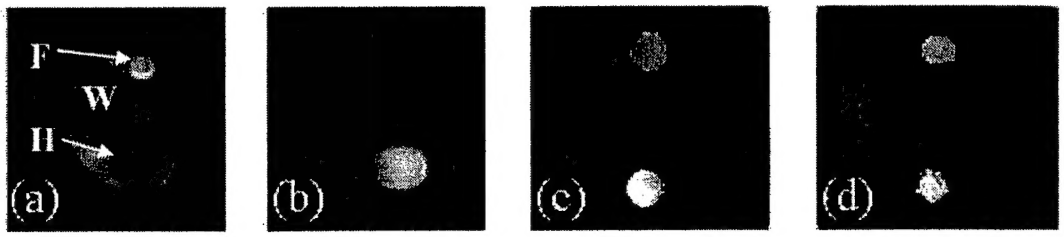
Introduction

There is increasing evidence for the importance of tissue oxygenation in development, progression, and response to cancer therapy. Oxygen is required for efficient function by most tissues and hypoxia leads to rapid cellular dysfunction and damage. In addition, hypoxic tumor cells are refractory to radiotherapy. Thus, the opportunity to measure tissue oxygen tension non-invasively may be significant in understanding mechanisms of tissue function and in clinical prognosis. The linear dependence of R_1 of fluorocarbon ^{19}F resonances on pO_2 is well known and has been studied extensively. We have studied the potential of HMDSO as a ^{1}H based pO_2 reporter molecule. In a related study (submitted to ISMRM 2004) data is presented for the linear dependence of R_1 of HMDSO on pO_2 (R_1= 0.13+ 0.00175*pO2[torr] at 37^{o}C) and the relative insensitivity of R_1 to temperature fluctuations and presence of physiologically relevant metal ions. Here we demonstrate the implementation of an imaging protocol (suitable for future in vivo studies) on phantoms.

Materials and Methods

The data was acquired on a Varian 4.7T scanner. A spin-echo EPI based pulse sequence was used for imaging and measuring T_1 values. The sequence consisted of a) a train of 20 non-selective 90^{o} pulses for saturation of signal followed by a delay *tau* for magnetization recovery, b) 3 CHESS[1] pulses for frequency selective saturation of water and fat immediately followed by c) spin-echo EPI detection with a slice selective 90^{o} pulse and a frequency selective 180^{o} pulse. T_1 values were measured using this sequence with the ARDVARC (*A*lternating *R*elaxation *D*elays with *V*ariable *A*cquisitions for *R*eduction of *C*learance effects) protocol[2]. For comparison, reference images were obtained using a spin echo sequence. T_1 maps were made using the Varian Image Browser software.

Results and Discussion



a) *T_1 weighted spin-echo image of phantom with smaller tubes containing mineral oil (F) and HMDSO (H) inside a tube containing water (W) and* ***b)*** *proton density weighted EPI image of the same phantom with fat and water suppression. T_1 maps of a phantom containing HMDSO saturated with gases with different concentrations of oxygen obtained by* ***c)*** *spin-echo sequence and* ***d)*** *the spin-echo EPI sequence.*

HMDSO has a single proton resonance with a chemical shift of –5ppm relative to water. Since our application is oriented towards T1 relaxometry, our approach to suppression involves frequency selective pulses. The EPI based sequence with frequency selective excitation and suppression was demonstrated to effectively suppress signals from mineral oil (to simulate fat) and water. The choice of a long echo time (>100ms) enabled suppression of any residual fat signal, resulting from its proximity to the HMDSO resonance. T1 data was obtained within 2 and half minutes. This approach is tailored towards future in vivo applications and the short acquisition time can allow us to monitor dynamic response to oxygen challenge. Minimal toxicity and wide availability add to the promise of HMDSO as a pO2 reporter molecule.

Acknowledgements

This work was supported by NCI Pre-ICMIC P20 CA086354 and DOD DAMD17-03-1-0101

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